# Inflammation and Asymmetric Dimethylarginine for Predicting Death and Cardiovascular Events in ESRD Patients

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## Summary

**Background** Endothelial dysfunction as assessed by asymmetric dimethylarginine (ADMA) and inflammation has been consistently linked to atherosclerosis, death, and cardiovascular (CV) events in ESRD patients. Inflammation amplifies the effect of ADMA on the severity of atherosclerosis in ESRD patients, but it is still unknown whether inflammation and ADMA interact in the high risk of death and CV events in this population.

**Design, setting, participants, & measurements** In a cohort of 225 hemodialysis patients, we investigated the interaction between inflammatory biomarkers (C-reactive protein and IL-6) and ADMA as predictors of death and CV events over an extended follow-up (13 years).

**Results** During follow-up, 160 patients died, and 123 had CV events. With crude and multiple Cox regression analyses, an interaction was found between inflammation biomarkers and ADMA for explaining death and CV events in ESRD patients. The adjusted hazard ratios (HRs) for death (HR, 2.18; 95% confidence interval [CI], 1.34 to 3.54) and CV outcomes (HR, 2.59; 95% CI, 1.47 to 4.55) of patients with C-reactive protein and ADMA above the median were higher than expected in the absence of interaction under the additive model (1.15 and 1.97, respectively) and significantly higher than in patients with only one biomarker above the median. Data analyses carried out by stratifying patients according to IL-6 provided similar results.

**Conclusions** These data support the hypothesis that inflammation amplifies the risk of death and CV events associated with high ADMA levels in ESRD. These analyses further emphasize the need for intervention studies to attenuate inflammation and high ADMA levels in this population.

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## Introduction

Over the last decade substantial evidence has been accrued that asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric-oxide synthase, is consistently related to cardiovascular (CV) complications and adverse clinical outcomes in disparate populations (1–14). Indeed, high ADMA was associated with a high risk of death in ESRD patients (2–5) as well as in patients with chronic kidney disease (8,9), with peripheral artery disease (10), with coronary artery disease (11), and in the Framingham offspring study (14). Although it remains uncertain whether the association between ADMA and clinical outcomes is causal in nature (15), a variety of experimental and clinical studies implicate high ADMA in CV remodeling and dysfunction in ESRD patients (16,17). ADMA infusion impairs vascular reactivity in healthy subjects (18), and high levels of this methylarginine are associated with endothelial dysfunction in untreated, uncomplicated, essential hypertensives (19). High ADMA was associated with enlarged atrial volume in the Framingham heart study (20) and with concentric left ventricular hypertrophy in ESRD patients (16). By the same token, ADMA emerged as a robust correlate of intima-media thickness both in the general population (21) and in ESRD patients (17).

Several lines of evidence indicate that endothelial dysfunction is intimately associated with chronic inflammation (22,23). In a prospective cohort study in patients with type 2 diabetes, an interaction emerged between inflammation (as assessed by circulating levels of C-reactive protein [CRP]) and circulating ADMA for explaining CV events (24). Although the independent prognostic value of ADMA and CRP is well established in ESRD patients (2,5,25–29), the possibility that these two major risk factors in dialysis patients may interact in explaining death and adverse CV outcomes has never been investigated. The issue

  

is of importance because in a previous longitudinal study in the dialysis population, we found a strong interaction between CRP and ADMA in predicting worsening in carotid atherosclerosis in these patients (17).

With this background in mind, we investigated the interaction between inflammatory biomarkers (CRP and IL-6) and ADMA levels for explaining long term mortality and CV events in ESRD patients within the Cardiovascular Risk Extended Evaluation in Dialysis (CREED) patients study cohort.

## Materials and Methods

### Protocol

The protocol was in conformity with the ethical guidelines of our institutions, and informed consent was obtained from each participant. All of the blood samples for laboratory tests were taken during a mid-week nondialysis day, between 8 a.m. and 1 p.m.

### Study Cohort

We studied an incident-prevalent cohort of 225 patients on chronic hemodialysis (123 men and 102 women) who had been on regular dialysis treatment for at least 6 months (median duration of regular dialysis treatment, 43 months; interquartile range, 21 to 109 months). The enrollment criteria in this cohort were: no history of congestive heart failure (defined as dyspnea in addition to two of the following conditions: raised jugular venous pressure, bibasilar crackles, pulmonary venous hypertension, or interstitial edema on chest x-ray, requiring hospitalization or extra ultrafiltration), left ventricular ejection fraction >35%, and no intercurrent or terminal illnesses. Hemodialysis patients were treated thrice weekly with standard bicarbonate dialysis (138 mmol/L sodium, 35 mmol/L $HCO_3$, 1.5 mmol/L potassium, 1.25 mmol/L calcium, 0.75 mmol/L magnesium) either with cuprophan or semisynthetic membranes (dialysis filter surface area, 1.1 to 1.7 $m^2$). The average urea Kt/V in these patients was 1.21 ± 0.26. Out of 225 patients, 113 (50%) had had one or more CV comorbidities: myocardial infarction in 25 patients, stroke in 20 patients, transient ischemic attacks in 25 patients, anginal episodes in 75 patients, peripheral vascular disease in 35 patients, and arrhythmia in 16 patients. Mortality and CV outcomes as related to ADMA over a shorter follow-up (4 to 5 years) in this cohort were published elsewhere (2,25).

### Follow-up

After the initial assessment, patients were followed up for 13 years. During the follow-up, CV events (electrocardiogram and cardiac enzyme documented myocardial infarction and anginal episodes and heart failure, whereas electrocardiogram documented arrhythmia, transient ischemic attacks, stroke, and other thrombotic events) and death were accurately recorded. Only 13 patients out of 225 (that is 6%) were lost to the follow-up. Each death was reviewed and assigned an underlying cause by a panel of five physicians. As a part of the review process, all of the available medical information about each death was collected. This information always included study and hospitalization records. In the case of an out-of-hospital death, family members were interviewed by telephone to better ascertain the circumstances surrounding death.

### Laboratory Measurements

Blood sampling was performed after an overnight fast between 8.00 a.m. and 1 p.m. always during a mid-week nondialysis day for hemodialysis patients. After 20 to 30 minutes of the patient resting quietly in a semirecumbent position, samples were taken into chilled EDTA vacutainers, placed immediately on ice, and centrifuged within 30 minutes at −4°C, and the plasma was stored at −80°C before assay. Serum lipids, albumin, calcium, phosphate, and hemoglobin measurements were made using standard methods in the routine clinical laboratory. The plasma concentrations of ADMA, CRP, and homocysteine were measured according to standard methods described elsewhere (2). It should be noted that the ADMA data were obtained in 2000 by HPLC (2). At that time, there were still no human plasma reference samples for absolute quantification available, which explains the fact that in most historic studies, ADMA values exceed the values obtained by today's gold standard methods by a factor of two to three. Therefore, when interpreting the data, relative rather than absolute values should be considered. Using a very similar cohort, we were recently able to replicate the principal findings obtained with the CREED cohort (5). The outcome results obtained by the new and by the historic HPLC method were very similar. This indicates that despite the difference in absolute values, the correlations and clinical implications on the basis of relative differences in historic (high absolute) or recent (low absolute) ADMA values remain the same. Accordingly, we based these calculations on relative ADMA data (dichotomized at the median). Serum levels of IL-6 were measured *de novo* by ELISA with the use of Quantikine high sensitivity kits (R&D Systems Inc., Minneapolis, MN).

### Statistical Analyses

The data are expressed as the means ± SD, median, and interquartile range or as a percent frequency, and comparisons among groups were made by one-way ANOVA (continuous variables) or chi-squared test (dichotomic variables) (see last column in Table 1). The relationship between continuous variables was investigated by the Pearson product moment correlation coefficient ($r$) and $P$ value. Variables having a positively skewed distribution were log transformed ($\log_{10}$) before the correlation study.

The independent predictive value of ADMA, CRP, and IL-6 for death and CV events was analyzed by multiple Cox regression analysis. The effect modification of inflammation biomarkers (CRP and IL-6) on the relationship between ADMA and outcomes was investigated by dividing the study population into four categories according to the median values of ADMA and CRP (or IL-6). In relative terms, the effect modification of inflammation biomarkers on the predictive value of ADMA was investigated by multiple Cox regression analyses. In multivariate models, we included a categorical variable resulting from the combination of CRP/IL-6 and ADMA (as recodified according to corresponding median values) as well as Framingham

**Table 1. Main demographic, somatometric, and clinical characteristics of the study population**

| | Whole Cohort ($n$ = 225) | <7.4 mg/L CRP and <2.4 μmol/L ADMA ($n$ = 64) | >7.4 mg/L CRP and <2.4 μmol/L ADMA ($n$ = 47) | <7.4 mg/L CRP and >2.4 μmol/L ADMA ($n$ = 50) | >7.4 mg/L CRP and >2.4 μmol/L ADMA ($n$ = 64) | $P$ |
|---|---|---|---|---|---|---|
| Age (years) | 60 ± 15 | 59 ± 17 | 65 ± 11 | 56 ± 14 | 59 ± 15 | 0.02 |
| Dialysis vintage (months) | 42 (21 to 109) | 27 (14 to 61) | 43 (25 to 77) | 86 (23 to 172) | 46 (24 to 129) | <0.001 |
| Male sex, $n$ (%) | 123 (55%) | 34 (53%) | 21 (45%) | 24 (51%) | 43 (67%) | 0.10 |
| Smokers, $n$ (%) | 83 (37%) | 21 (33%) | 15 (32%) | 19 (40%) | 28 (44%) | 0.48 |
| Diabetics, $n$ (%) | 34 (15%) | 9 (14%) | 11 (23%) | 3 (6%) | 11 (17%) | 0.14 |
| With CV comorbidities, $n$ (%) | 113 (50%) | 24 (38%) | 31 (66%) | 17 (36%) | 40 (63%) | <0.001 |
| On anti-hypertensive treatment, $n$ (%) | 82 (36%) | 20 (31%) | 12 (26%) | 19 (40%) | 30 (47%) | 0.09 |
| Systolic pressure (mmHg) | 139 ± 25 | 137 ± 25 | 142 ± 30 | 139 ± 26 | 141 ± 21 | 0.62 |
| Diastolic pressure (mmHg) | 76 ± 13 | 77 ± 13 | 76 ± 13 | 75 ± 12 | 76 ± 13 | 0.94 |
| Cholesterol (mg/dl) | 208 ± 58 | 192 ± 44 | 217 ± 72 | 218 ± 60 | 209 ± 55 | 0.06 |
| Hemoglobin (g/L) | 106 ± 19 | 110 ± 19 | 106 ± 15 | 109 ± 19 | 105 ± 20 | 0.32 |
| Calcium phosphate ($mmol^2/L^2$) | 4.52 ± 1.12 | 4.59 ± 1.19 | 4.48 ± 1.11 | 4.41 ± 1.04 | 4.57 ± 1.14 | 0.82 |
| Albumin (g/L) | 42 ± 5 | 43 ± 4 | 41 ± 4 | 42 ± 5 | 41 ± 6 | 0.14 |
| CRP (mg/L) | 7.5 (3.4 to 16.4) | 3.4 (3.4 to 3.5) | 20.6 (13.7 to 30.7) | 3.4 (3.4 to 4.3) | 15.7 (11.5 to 27.7) | <0.001 |
| IL-6 (pg/ml) | 5.0 (2.7 to 9.2) | 3.3 (2.6 to 5.3) | 4.8 (2.9 to 10.2) | 4.4 (2.2 to 8.2) | 7.7 (4.9 to 11.8) | <0.001 |
| ADMA (μmol/L) | 2.52 (1.58 to 3.85) | 1.63 (1.06 to 1.98) | 1.56 (1.10 to 1.97) | 3.55 (2.89 to 4.69) | 3.99 (3.33 to 5.24) | <0.001 |
| Homocysteine (μmol/L) | 27.0 (19.4 to 42.7) | 29.3 (20.3 to 52.0) | 25.4 (20.0 to 38.5) | 26.2 (18.2 to 44.7) | 27.3 (19.4 to 42.7) | 0.41 |

The data are expressed as the means ± SD, median and interquartile range, or as the percent frequency, as appropriate. The patients were grouped according to the median values of CRP and ADMA. Comparisons among groups were made by one-way ANOVA (continuous variables) or chi-squared test (dichotomic variables) (see last column). CRP, C-reactive protein; CV, cardiovascular; ADMA, asymmetric dimethylarginine.

risk factors (age, sex, smoking, diabetes, cholesterol, and systolic pressure), CV comorbidities, anti-hypertensive treatment, factors peculiar to ESRD (dialysis vintage, albumin, hemoglobin, and calcium phosphate), and homocysteine. Interaction (synergism) between CRP (or IL-6) and ADMA was defined as a deviation from additivity (30) occurring when the observed hazard ratio (HR) for study outcomes of patients with both high CRP (or high IL-6) and high ADMA was higher than that expected by summing up the hazard ratio of those with high CRP (or high IL-6) and low ADMA or low CRP (or low IL-6) and high ADMA minus one. The excess risk from both exposures in the presence of interaction relative to the excess risk from both exposures in the absence of interaction was assessed by calculating the synergy index (30). The proportionality assumption of Cox models was tested by the analysis of Schoenfeld residuals, and no violation was found. The homogeneity of HRs over time associated with key variables (ADMA, CRP, and IL-6 and their interaction) was investigated by analyzing the interaction of these variables and time. The data are expressed as HR, 95% confidence intervals, and *P* values. All of the calculations were done by a standard statistical package (SPSS for Windows, version 9.0.1, SPSS; Chicago, IL).

## Results

The main demographic and clinical characteristics of patients included in the study are detailed in Table 1. The prevalence of diabetes mellitus in this cohort was 15% (*i.e.* 34 patients out of 225). Eighty-three patients were habitual smokers (median, 20 cigarettes/d; interquartile range, 10 to 30 cigarettes/d). One hundred and twenty-two patients were on treatment with erythropoietin, and 82 patients were being treated with anti-hypertensive drugs (58 on monotherapy with angiotensin-converting enzyme inhibitors, angiotensin-1 antagonists, calcium channel blockers, $\alpha$- and $\beta$-blockers; and 24 on double or triple therapy with various combinations of these drugs).

**Table 2. Cox regression analyses of the main effect of ADMA, CRP, and IL-6 on the incidence rate of mortality and fatal and nonfatal CV events**

| Variables (units of increase) | Hazard Ratio (95% CI) and *P* Value | |
|---|---|---|
| | ADMA and CRP-based Model | ADMA and IL-6-based Model |
| All-cause mortality | | |
| age (1 year) | 1.04 (1.02 to 1.06), $P < 0.001$ | 1.04 (1.02 to 1.05), $P < 0.001$ |
| male gender | 1.67 (1.07 to 2.61), $P = 0.02$ | 1.55 (0.99 to 2.41), $P = 0.06$ |
| smoking | 1.22 (0.80 to 1.86), $P = 0.36$ | 1.24 (0.81 to 1.89), $P = 0.32$ |
| diabetes | 2.26 (1.45 to 3.51), $P < 0.001$ | 2.42 (1.55 to 3.78), $P < 0.001$ |
| cholesterol (20 mg/dl) | 1.01 (0.94 to 1.08), $P = 0.81$ | 1.02 (0.95 to 1.09), $P = 0.64$ |
| systolic pressure (1 mmHg) | 1.00 (0.99 to 1.01), $P = 0.006$ | 1.00 (0.99 to 1.01), $P = 0.94$ |
| CV comorbidities (0 = no; 1 = yes) | 1.65 (1.16 to 2.36), $P = 0.006$ | 1.57 (1.10 to 2.23), $P = 0.01$ |
| anti-hypertensive treatment (0 = no; 1 = yes) | 0.89 (0.60 to 1.31), $P = 0.56$ | 0.87 (0.59 to 1.29), $P = 0.50$ |
| dialysis vintage (10 months) | 1.00 (0.98 to 1.03), $P = 0.98$ | 1.00 (0.97 to 1.02), $P = 0.87$ |
| albumin (1 g/L) | 0.97 (0.93 to 1.01), $P = 0.14$ | 0.98 (0.94 to 1.02), $P = 0.27$ |
| hemoglobin (1 g/L) | 1.01 (0.99 to 1.02), $P = 0.08$ | 1.01 (0.99 to 1.02), $P = 0.11$ |
| calcium phosphate (1 $mmol^2/L^2$) | 1.04 (0.89 to 1.21), $P = 0.66$ | 1.06 (0.91 to 1.24), $P = 0.47$ |
| homocysteine (10 $\mu$mol/L) | 1.04 (0.98 to 1.10), $P = 0.21$ | 1.03 (0.98 to 1.09), $P = 0.22$ |
| ADMA (1 $\mu$mol/L) | 1.22 (1.12 to 1.34), $P < 0.001$ | 1.21 (1.10 to 1.32), $P < 0.001$ |
| CRP (5 mg/L) | 1.04 (1.01 to 1.07), $P = 0.008$ | |
| IL-6 (1 pg/ml) | | 1.03 (1.01 to 1.05), $P = 0.002$ |
| Fatal and nonfatal CV events | | |
| age (1 year) | 1.03 (1.02 to 1.06), $P < 0.001$ | 1.03 (1.02 to 1.06), $P < 0.001$ |
| male gender | 1.36 (0.82 to 2.27), $P = 0.23$ | 1.34 (0.80 to 2.24), $P = 0.27$ |
| smoking | 1.59 (0.99 to 2.56), $P = 0.06$ | 1.61 (1.00 to 2.58), $P = 0.05$ |
| diabetes | 1.92 (1.16 to 3.18), $P = 0.01$ | 1.94 (1.17 to 3.23), $P = 0.01$ |
| cholesterol (20 mg/dl) | 1.04 (0.96 to 1.12), $P = 0.32$ | 1.04 (0.96 to 1.12), $P = 0.31$ |
| systolic pressure (1 mmHg) | 1.01 (0.99 to 1.02), $P = 0.86$ | 1.00 (0.99 to 2.58), $P = 0.87$ |
| CV comorbidities (0 = no; 1 = yes) | 1.56 (1.03 to 2.35), $P = 0.04$ | 1.55 (1.02 to 2.34), $P = 0.04$ |
| anti-hypertensive treatment (0 = no; 1 = yes) | 2.02 (1.31 to 3.12), $P = 0.002$ | 2.00 (1.29 to 3.09), $P = 0.002$ |
| dialysis vintage (10 months) | 1.03 (0.99 to 1.06), $P = 0.06$ | 1.03 (0.99 to 1.06), $P = 0.06$ |
| albumin (1 g/L) | 0.98 (0.94 to 1.03), $P = 0.48$ | 0.98 (0.94 to 1.03), $P = 0.52$ |
| hemoglobin (1 g/L) | 1.01 (0.99 to 1.02), $P = 0.28$ | 1.01 (0.99 to 1.02), $P = 0.30$ |
| calcium phosphate (1 $mmol^2/L^2$) | 1.05 (0.88 to 1.25), $P = 0.60$ | 1.05 (0.89 to 1.25), $P = 0.56$ |
| homocysteine (10 $\mu$mol/L) | 1.02 (0.96 to 1.09), $P = 0.51$ | 1.02 (0.96 to 1.09), $P = 0.52$ |
| ADMA (1 $\mu$mol/L) | 1.18 (1.07 to 1.30), $P = 0.001$ | 1.17 (1.06 to 1.29), $P = 0.001$ |
| CRP (1 mg/L) | 1.01 (0.97 to 1.06), $P = 0.61$ | — |
| IL-6 (1 pg/ml) | — | 1.01 (0.98 to 1.03), $P = 0.60$ |

CRP, C-reactive protein; CV, cardiovascular; ADMA, asymmetric dimethylarginine; CI, confidence interval.

### ADMA, CRP, and IL-6 Levels in the Study Population

The circulating levels of ADMA (median, 2.4 μmol/L; interquartile range, 1.6 to 3.8 μmol/L), CRP (median, 7.4 mg/L; interquartile range, 3.4 to 16.4 mg/L), and IL-6 (median, 5.0 pg/ml; interquartile range, 2.7 to 9.2 pg/ml) were above the upper limit of the corresponding normal ranges in 133 (59%), 124 (55%), and 32 (14%) ESRD patients, respectively. On univariate analyses, the plasma levels of ADMA were significantly related to those of CRP ($r = 0.13$, $P = 0.046$) and IL-6 ($r = 0.18$, $P = 0.009$). A stratified analysis according to the median values of CRP (below/above, 7.4 mg/dl) and ADMA (below/above, 2.4 μmol/L) (Table 1) showed that age ($P = 0.02$), dialysis vintage ($P < 0.001$), and the proportion of patients with CV comorbidities ($P < 0.001$) significantly differed among groups, whereas cholesterol ($P = 0.06$) and the proportion of patients treated with anti-hypertensive drugs ($P = 0.09$) just failed to reach statistical significance. Similar results were obtained by stratifying patients according to ADMA and IL-6 (below/above 5 pg/ml) (data not shown).

### Effect Modification of Inflammation on the ADMA-Outcome Relationship

During the follow-up period (156 months), 160 patients died (15 deaths/100 person-years), and 123 had fatal and nonfatal CV events (13 events/100 person-years). In Cox models including ADMA and CRP (or IL-6) as well as a series of potential confounders (Table 2), these biomarkers significantly predicted death (ADMA, $P < 0.001$; CRP, $P = 0.008$; IL-6, $P = 0.002$), whereas ADMA ($P = 0.001$) was the sole biomarker explaining fatal and nonfatal CV events (Table 2). The relationship between ADMA and the incidence rate of mortality and CV outcomes was closely dependent on CRP categories (effect modification of ADMA by CRP) (Figure 1), the incidence rate of mortality and CV events being maximal in patients with high ADMA (≥2.4 μmol/L) and high CRP (≥7.4 mg/dl) and minimal in patients with low ADMA (<2.4 μmol/L) and low CRP (<7.4 mg/dl), and this was also true when the same analysis was carried out according to IL-6 (Figure 1). Of note, in patients with low levels of CRP or IL-6, no excess risk for death and CV events was associated with high levels of ADMA (Figure 1). After data adjustment for potential confounders, patients with high circulating levels of ADMA and low CRP (or IL-6) did not show an increased risk of death (Figure 2) but tended to have an enhanced hazard ratio for fatal and nonfatal CV events (Figure 3) Accordingly, in multiple Cox regression models (Figures 2 and 3), an interaction was found between inflammation biomarkers and ADMA for explaining all-cause mortality and fatal and nonfatal CV events. Indeed, the adjusted hazard ratios for death (Figure 2) and CV outcomes (Figure 3) of patients

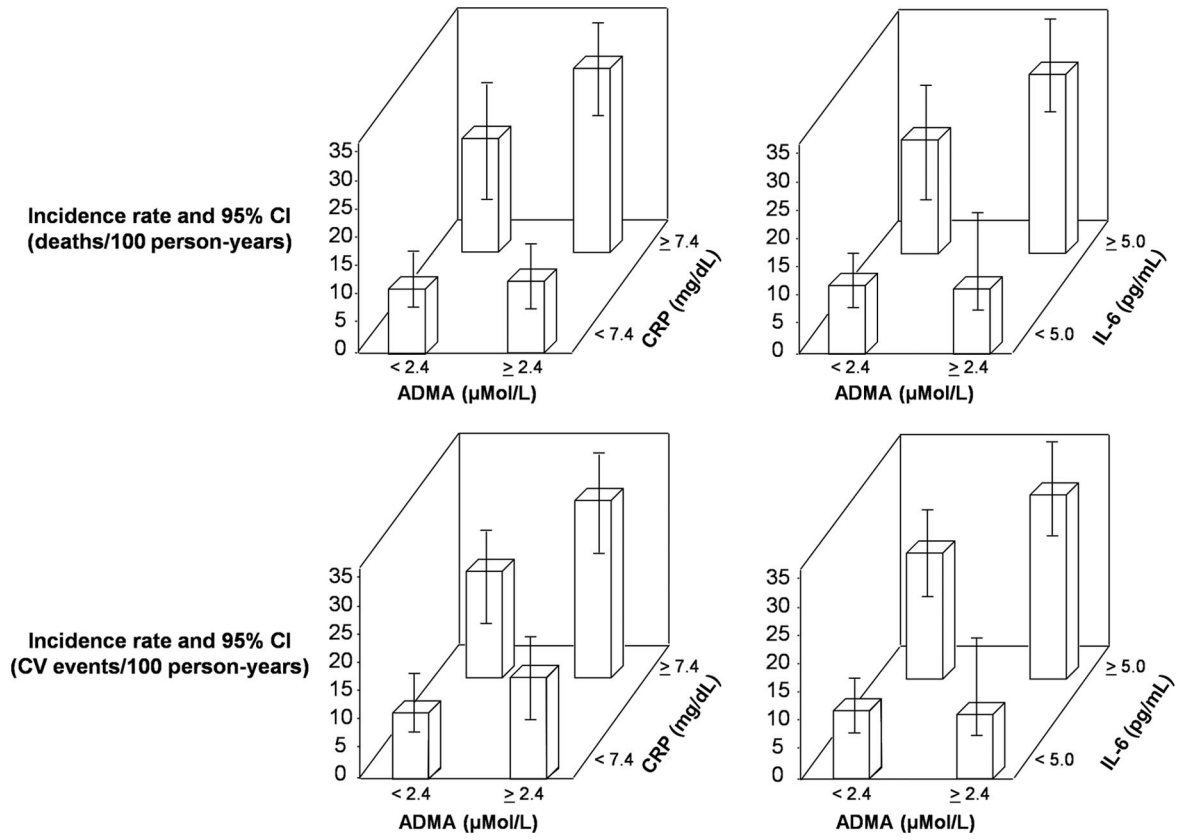


**Figure 1. | Relationship of asymmetric dimethylarginine (ADMA), C-reactive protein (CRP), and IL-6 (as recodified according to the corresponding median value) and study outcomes (all-cause mortality and fatal and nonfatal cardiovascular [CV] events).** The data are crude (unadjusted) incidence rates and 95% confidence intervals (CIs). The patients are divided into four groups according to the median values of ADMA and CRP (or IL-6).

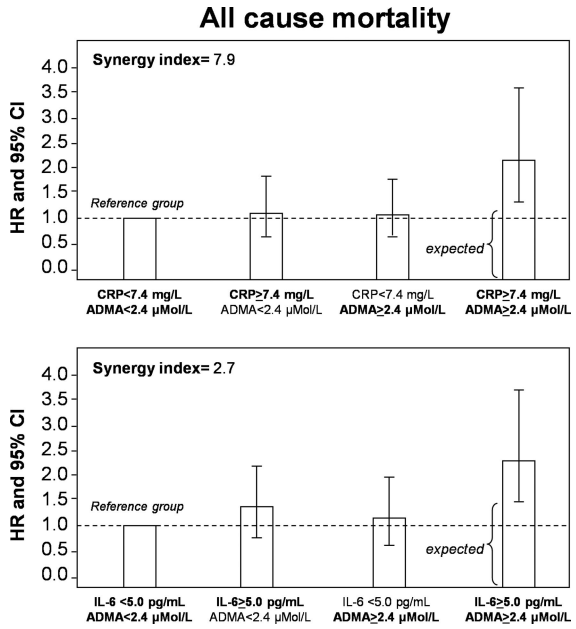


**Figure 2. | Interaction between C-reactive protein (CRP), IL-6, and asymmetric dimethylarginine (ADMA) (below/above the corresponding median values) for explaining all-cause mortality.** The data are expressed as hazard ratios (HR) and 95% confidence intervals (CI). The data were adjusted for age, sex, smoking, diabetes, cholesterol, systolic pressure, cardiovascular (CV) comorbidities, antihypertensive treatment, dialysis vintage, albumin, hemoglobin, calcium phosphate, and homocysteine. Deviation from additivity or the presence of interaction (synergism) was assessed by comparing the observed joint effect of high CRP (or IL-6) and high ADMA levels with that expected in the absence of interaction (see below). For the expected effect of CRP and ADMA in the absence of interaction, $HR_{ADMA\ <2.4\ \mu mol/L\ and\ CRP\ \geq 7.4\ mg/L} + HR_{ADMA\ \geq 2.4\ \mu mol/L\ and\ CRP\ <7.4\ mg/L} - 1 = 1.08 + 1.07 - 1 = 1.15$. For the expected effect of IL-6 and ADMA in the absence of interaction, $HR_{ADMA\ <2.4\ \mu mol/L\ and\ IL\text{-}6\ \geq 5.0\ mg/L} + HR_{ADMA\ \geq 2.4\ \mu mol/L\ and\ IL\text{-}6\ <\ 5.0\ mg/L} - 1 = 1.36 + 1.12 - 1 = 1.48$.

with increased ADMA and CRP were higher than those expected in the absence of interaction under the additive model and significantly higher ($P < 0.001$) than those in patients with only one biomarker increased (Figures 2 and 3). Data analysis carried out by stratifying patients according to IL-6 provided similar results ($P < 0.02$) (Figures 2 and 3). Remarkably, the excess risk for death and CV events caused by the interaction (synergy index) was from 1.6 to 7.9 times higher than that portended by high ADMA and high inflammation markers in the absence of interaction (Figures 2 and 3).

The risk excess for death and fatal and nonfatal CV events associated with high circulating levels of ADMA and inflammatory biomarkers (CRP and IL-6) did not change throughout the follow-up period ($P > 0.20$), implying that the effect modification of CRP and IL-6 on the relationship between ADMA and study outcomes remained stable and statistically significant over time.

## Discussion

This study shows that, independently of traditional and nontraditional risk factors, inflammation amplifies the risk of death and CV events associated with high plasma ADMA in dialysis patients and that in patients with relatively low CRP and/or IL-6, no excess risk for death and CV events is associated with high levels of ADMA.

### Inflammation and Endothelial Dysfunction in ESRD Patients

Inflammation in ESRD patients is a multi-factorial problem (22). Both dialysis-related and dialysis-independent factors may promote inflammation by stimulating the synthesis and/or release of several pro-inflammatory cytokines like CRP, IL-1, IL-6, TNF-α, and IFN-γ (23). Impaired endothelium-dependent vasodilation is a hallmark in patients with ESRD (31), and high ADMA levels are considered as a major factor in endothelial dysfunction in chronic renal failure (31,32). In our study, the majority of ESRD patients had circulating levels of ADMA and CRP above the corresponding upper limit of the normal range (59% and 55%, respectively). Circulating levels of ADMA were directly and significantly related to those of CRP and IL-6, indicating that inflammation and endothelial dysfunction are parallel processes in ESRD patients. These relationships may

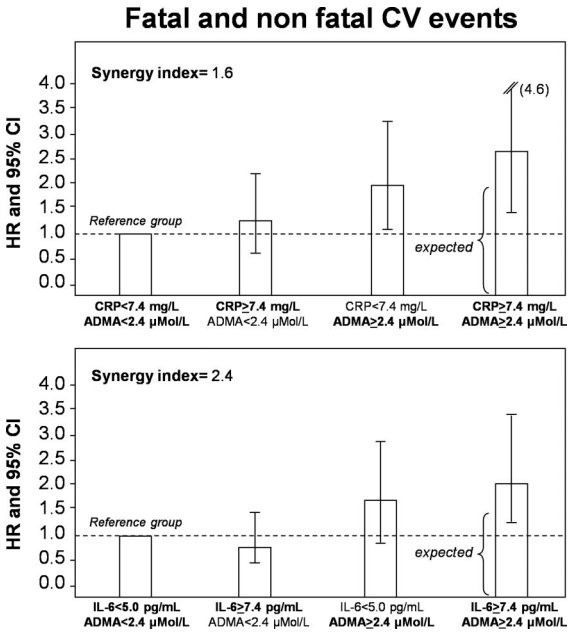


**Figure 3. | Interaction between C-reactive protein (CRP), IL-6, and asymmetric dimethylarginine (ADMA) (below/above the corresponding median values) for explaining fatal and nonfatal cardiovascular (CV) events.** The data are expressed as hazard ratios (HR) and 95% confidence intervals (CI). The data were adjusted for age, sex, smoking, diabetes, cholesterol, systolic pressure, CV comorbidities, anti-hypertensive treatment, dialysis vintage, albumin, hemoglobin, calcium phosphate, and homocysteine. Deviation from additivity or presence of interaction (synergism) was assessed by comparing the observed joint effect of high CRP (or IL-6) and high ADMA levels with that expected in the absence of interaction (see below). For the expected effect of CRP and ADMA in the absence of interaction, $HR_{ADMA\ <2.4\ \mu mol/L\ and\ CRP\ \geq 7.4\ mg/L} + HR_{ADMA\ \geq 2.4\ \mu mol/L\ and\ CRP\ <7.4\ mg/L} - 1 = 1.13 + 1.84 - 1 = 1.97$. For the expected effect of IL-6 and ADMA in the absence of interaction, $HR_{ADMA\ <2.4\ \mu mol/L\ and\ IL\text{-}6\ \geq 5.0\ mg/L} + HR_{ADMA\ \geq 2.4\ \mu mol/L\ and\ IL\text{-}6\ <5.0\ mg/L} - 1 = 0.78 + 1.64 - 1 = 1.42$.

reflect a causal link, because in patients with familial Mediterranean fever, both ADMA and CRP levels show a concomitant increase during the acute phase of the disease to revert to normal after the resolution of fever (33).

### Interaction between ADMA and Inflammation

The relationship between high ADMA and CRP is context dependent. Indeed, in acute sepsis, ADMA is downregulated (34), whereas high ADMA levels are consistently associated with biomarkers of inflammation in a variety of chronic conditions including untreated essential hypertension (35), glucose intolerance (36), familial Mediterranean fever (33), and inflammatory bowel diseases (37). *In vitro*, ADMA induces TNF-$\alpha$ production via reactive oxygen species/NF-$\kappa$B-dependent pathway (38). On the other hand, the generation of reactive oxygen species, an important initial event in inflammation, inhibits the enzyme that degrades ADMA (dimethylarginine dimethylaminohydrolase), facilitating local and/or systemic ADMA accumulation. ADMA in turn increases the generation of the downstream pro-inflammatory mediators TNF-$\alpha$ and IL-8 and activates the NF-$\kappa$B pathway and the binding of monocytes to endothelial cells (39). Even although an independent prognostic value of ADMA and inflammation was described in the same cohort over a much shorter (4 to 5 years *versus* 13 years) follow-up (2,25), the interaction between this methylarginine and inflammation for explaining death and CV events has never been tested. In this study, patients with both high ADMA and CRP (or high IL-6) levels exhibited an increased risk of death and fatal and nonfatal CV events compared with those with only one elevated biomarker, and such an excess risk exceeded what one would expect by adding the individual risks of these factors (synergism). This finding is in keeping with the results of a longitudinal study by our group (17) in which we found a synergic interaction between CRP and ADMA to explain the progression of carotid atherosclerosis in ESRD patients (17). In the AURORA study (40), treatment with Rosuvastatin reduced CRP, but this drug had no effect on the incidence rate of death and CV events in ESRD patients. Rosuvastatin is one of the most potent ADMA modifiers, and improvement in endothelial dysfunction in patients with hypercholesterolemia goes along with the ADMA-lowering effect of this drug (41). Secondary analyses in AURORA restricted to patients with high ADMA and high CRP (that is, in the subgroup of patients in whom, as suggested by our findings, the beneficial effect of Rosuvastatin might be maximized) may allow preliminary testing the hypothesis generated by this study. The main limitation of our study is its observational nature, which precludes the possibility of drawing definitive conclusions about the nature (causal/non-causal) of the relationships we found. The strengths of our hypothesis-generating study are the adequate sample size, the robustness of the statistical approach, the internal coherence, and biologic plausibility of the results.

## Conclusions

Inflammation and endothelial dysfunction as assessed by circulating levels of ADMA have an independent, synergic effect for explaining all cause mortality and fatal and nonfatal CV events in ESRD patients. These results generate the hypothesis that interventions aimed at attenuating/preventing endothelial dysfunction and inflammation may attenuate the exceedingly high CV risk burden of these patients.

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### Disclosures

None.

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